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EXPERT COMMITTEE ON BIOLOGICAL STANDARDIZATION
Geneva, 17 to 21 October 2011

Recommendations to Assure the Quality, Safety and Efficacy of BCG Vaccines

Proposed replacement of: TRS 745, Annex 2 and Amendment to TRS 771, Annex 12

This document has been prepared for the purpose of inviting comments and suggestions on the proposals contained therein, which will then be considered by the Expert Committee on Biological Standardization (ECBS). **The text in its present form does not necessarily represent an agreed formulation of the Expert Committee. Comments proposing modifications to this text MUST be received by 23 September 2011** and should be addressed to the World Health Organization, 1211 Geneva 27, Switzerland, attention: Quality Safety and Standards (QSS). Comments may also be submitted electronically to the Responsible Officer: Dr HyeNa Kang at email: kangh@who.int.

The outcome of the deliberations of the Expert Committee will be published in the WHO Technical Report Series. The final agreed formulation of the document will be edited to be in conformity with the "WHO style guide" (WHO/IMD/PUB/04.1).

Recommendations published by the WHO are intended to be scientific and advisory. Each of the following sections constitutes guidance for national regulatory authorities (NRAs) and for manufacturers of biological products. If a NRA so desires, these Recommendations may be adopted as definitive national requirements, or modifications may be justified and made by the NRA. It is recommended that modifications to these Recommendations be made only on condition that modifications ensure that the vaccine is at least as safe and efficacious as that prepared in accordance with the recommendations set out below. The parts of each section printed in small type are comments for additional guidance intended for manufacturers and NRAs, which may benefit from those details.

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Introduction

The last revision of the requirements for dried bacillus Calmette Guérin (BCG) vaccine for human use was in 1985, and an amendment which updated the section on the expiry date was published in 1988 (1, 2). Recent WHO consultation meetings (3, 4, 5, 6) have addressed the issues on improvement of vaccine characterization and quality control assays of BCG vaccine to reflect current state-of-the-art technology. In addition, a recommendation to replace the international reference preparation for BCG vaccine by sub-strain specific reference reagents evaluated by collaborative studies has been proposed. This guideline provides recommendations for the production and control of BCG vaccines in Part A, for preclinical evaluation in Part B, and for the content of the clinical development program applicable to BCG vaccines in Part C. The term of 'preclinical' evaluation applies for classical BCG vaccine products still in need of such evaluation, including the newly manufactured products requiring clinical trial studies. The clinical part of this document intends to provide a basis for assessment of efficacy and safety of BCG vaccines in pre-licensing clinical trials as well as in post-marketing surveillance, monitoring consistency of production and clinical testing of new classical BCG vaccine products. If important changes have been introduced to an authorized production process, the need for preclinical and clinical testing should be considered on a case-by-case basis in consultation with the NRA(s) concerned.

General considerations

Tuberculosis (TB) was declared a global emergency by the WHO in 1993, and *Mycobacterium tuberculosis* (*M. tuberculosis*) is now considered to be responsible for more adult deaths than any other pathogens. Vaccination with BCG still remains the standard for TB prevention in most countries because of its efficacy in preventing life-threatening forms of TB in infants and young children. It is inexpensive and usually requires only one administration in either newborn or adolescents (7, 8). As there is currently no suitable alternative, BCG will remain in use in the foreseeable future and may continue to be used as a prime vaccine in a Prime-Boost immunization schedule in conjunction with new TB vaccines (4).

BCG vaccine is a live attenuated vaccine originated from culturing *M. bovis* isolated from cattle and cultured for a period of 13 years and a total of 231 passages (7). The BCG vaccine was first used to immunize humans in 1921. Following its introduction into the WHO Expanded Programme on

Immunization (EPI) in 1974, the vaccine soon reached global coverage rates exceeding 80% in countries endemic for TB (9).

Over the years, different BCG vaccine seed strains have evolved from the original vaccine strain for production. A number of BCG vaccine strains that are used worldwide differ in terms of their genetic and phenotypic properties, and their reactogenicity and immunogenicity profile when given to infants and children. With this background of a diversity of sub-strains, manufacturing processes, immunization schedules and levels of exposure to environmental mycobacteria and virulent *M. tuberculosis* infection, different levels of protective efficacy of BCG vaccines in adult populations have been reported (10). However, the data are insufficient to make recommendations on whether one strain should be preferred over the other (11). The United Nations agencies are the largest supplier of BCG vaccines, distributing more than 120 million doses each year to more than 100 countries. Worldwide, the most commonly used vaccine strains are currently Danish 1331, Tokyo 172-1 and Russian BCG-I because they are supplied by United Nations Children's Fund (UNICEF) who purchases the vaccines through a published prequalification process which determines their eligibility for use in national immunization programmes (12).

There has been particular concern over the safety of BCG vaccination in human immunodeficiency virus (HIV)-infected subjects (8). WHO had previously recommended that in countries with a high burden of TB, a single administration of BCG vaccine should be given to all healthy infants as soon as possible after birth, unless the child presented a symptomatic HIV infection (9). However, recent evidence shows that children who were HIV-infected when vaccinated with BCG at birth, and who later developed AIDS, were at increased risk of developing disseminated BCG disease. Among these children, the benefits of potentially preventing severe TB are outweighed by the risks associated with the use of BCG vaccine; and the use of BCG vaccines at birth should follow the recommendations from WHO Strategic Advisory Group of Experts (SAGE) on immunization and position papers (13, 14).

Special considerations

The formulation of international requirements for freeze-dried BCG vaccine is complicated by the following: *(a)* a number of different sub-strains derived from the original strain of BCG are used in vaccine manufacture; *(b)* a number of different manufacturing and testing procedures are employed;

(c) difficulties of translation from significant differences *in vitro* and *in vivo* between different BCG vaccine strains to any possible differences in protective efficacy against TB in humans; *(d)* vaccines with different total bacterial content and number of culturable particles are produced; and *(e)* vaccines intended for administration by different routes are prepared.

Scope of the Recommendations

These revised recommendations refer to freeze-dried BCG vaccines prepared from sub- strains derived from original BCG for use in the prevention of TB. Where BCG vaccine is issued in liquid form, the application of these recommendations is entirely under the responsibility of the national regulatory authority (NRA). In that case, only the relevant parts of these requirements apply because limited stability of liquid BCG limits the possibility of completing the entire recommended control test schedule. Although many of the principles expressed in this document (*e.g.* manufacturing, quality control) are expected to apply also to new recombinant BCG and other live attenuated mycobacterial vaccines modified by molecular biology techniques, these novel vaccines are outside the scope of this guideline. The same pertains to the use of BCG for immunotherapy (*e.g.* treatment of bladder cancer). However, applicability of issues on preclinical and clinical evaluations should be considered on a case-by-case basis. These recommendations have been formulated primarily to cover vaccines intended for intradermal and percutaneous administration. Although WHO recommends intradermal administration of the vaccine, preferably in the deltoid region of the arm using syringe and needle, other administration methods such as percutaneous application by the multiple puncture technique are practiced in some countries (9, 15, 16, 17).

BCG vaccine strains

The original BCG vaccine strain was formerly distributed by the Pasteur Institute of Paris and sub-cultured in different countries using different culture conditions which were not standardized. Over the years, more than 14 sub-strains of BCG have evolved and been used as BCG vaccine strains in different parts of the world (see Appendix 1). Recently, the various sub-strains have been studied by comparative genomics (18, 19). BCG vaccine strains were thus divided into the “early” strains, in which the original characteristics of 'authentic Pasteur' were conserved with less deletions, insertions and mutation in the genome of the bacilli than the “late” strains. Such strains are represented by BCGs Russia BCG-I, Moreau-RJ, Tokyo 172-1, Sweden, and Birkhaug; and the “late” strains, such as BCGs Pasteur 1173P2, Danish 1331, Glaxo (Copenhagen 1077) and Prague. The genomic

sequences of BCG Pasteur 1173P2 as a "late" strain and BCG Tokyo 172-1 as an "early" strain were determined in 2007 and 2009, respectively (18, 19). There is insufficient direct evidence to suggest that various BCG sub-strains differ significantly in their efficacy to protect against TB in humans. However, evidence from animal and human studies indicates differences in the immune responses induced by different BCG vaccine strains (12). Although the "early" strains may confer better protection against TB in some animal studies (18, 20), commonly administered BCG vaccine strains including both evolutionary "early" and "late" strains induce comparable protective immunity against TB (21).

Only master seed lots that have been shown to be acceptable by laboratory and clinical tests on batches derived from them should be used for production of working seed lots and/ or final product. A suitable seed lot of BCG should yield vaccines that give protection in experimental animals, produce a relatively high level of immunological responses to *M. tuberculosis* antigens including tuberculin sensitivity in humans, and have an acceptably low frequency of adverse reactions (see A3.1).

Some manufacturers of freeze-dried BCG vaccine have modified their master seed lot strain to make it more suitable for their particular production procedure. The seed lots prepared in this way may not retain the same immunogenic properties, and should be used only with the approval of the NRA.

In practice, a product prepared from BCG seed lots may generally be investigated in humans only for their properties of producing tuberculin sensitivity and vaccination lesions. The former should be measured by the distribution of tuberculin reactions according to size in persons vaccinated with a given dose of BCG vaccine. A low dose of tuberculin should be employed (*e.g.* equivalent to 5 IU of the 1st International Standard for Purified Protein Derivative (PPD) of *M. tuberculosis*, or 2 tuberculin units (TU) of a batch of PPD RT23 with Tween 80).

Currently three sub-strains specific Reference Reagents for BCG vaccines are available and they are the BCG Danish 1331, Tokyo 172-1 and Russian BCG-I.

Potency-related tests

There is some evidence that BCG seed lots that have been shown to produce vaccines with protective potency in laboratory animals and tuberculin sensitivity in humans will give effective protection against TB in humans. It should be noted that tuberculin sensitivity is a marker for cell-mediated immune responses to mycobacteria and not a direct indicator of protective immunity. A number of alternative laboratory tests have been developed primarily for research purposes, but to date, none have been proven to be reliable indicators of protective immune-conversion following administration of different vaccines.

Field observations should be made in conjunction with laboratory studies in animals. The latter should include protection tests, tests of vaccination lesions, and tests for tuberculin conversion. Immunizing efficacy should be measured in terms of degree of protection afforded to the test animals against a challenge with virulent *M. tuberculosis.* Sensitizing efficacy should be measured by the average dose of vaccine that will convert a negative tuberculin reaction in guinea-pigs to a positive one, as well as by the reaction time that such conversion is effected. In these animal tests, the inclusion, for comparative purposes, of an in-house reference BCG vaccine prepared from a seed lot known to be effective in animals and humans is recommended.

As currently there is no biomarker, which directly correlates to clinical efficacy of BCG vaccine, the laboratory tests at present in use and included in these requirements are designed to ensure that new vaccine lots do not differ appreciably from those that have already been shown to be safe and effective in humans as regards their ability to induce adequate sensitivity to tuberculin, or from an in-house reference vaccine prepared from a seed lot shown to be safe and effective in humans. At present, for batch control purposes, much reliance is placed on tests for the estimation of the total bacterial content and for the number of culturable particles. It is not possible to specify single requirements for the total bacterial content and for the number of culturable particles for all vaccines (22), since different sub-strains and methods of manufacture may yield different specifications for these parameters. For example, although the number of culturable bacteria in a single human dose may differ for different vaccines, these vaccines may show satisfactory properties as regards their ability to induce adequate sensitivity to tuberculin and their safety in humans. It is therefore essential that clinical studies for dose optimisation in humans be carried out to estimate suitable total bacterial contents and the number of culturable particles for a particular manufacturer's product. For a particular vaccine, the difference between the lower and upper specification for the number of

culturable particles should not be larger than 4-fold. In addition, it is necessary to perform animal experiments that give an indication of the safety and efficacy of the vaccines to the satisfaction of the NRA.

Part A. Manufacturing recommendations

A.1 Definitions

A.1.1 *International name and proper name*

The international name should be "Freeze-dried BCG vaccine". The proper name should be the equivalent of the international name in the language of the country of origin. The use of the international name should be limited to vaccines that satisfy the recommendations formulated below.

A.1.2 *Descriptive definition*

Freeze-dried BCG vaccine is a freeze-dried preparation containing live bacteria derived from a culture of the bacillus of Calmette and Guérin, known as BCG, intended for intradermal injection. The name of the freeze-dried vaccine intended for percutaneous vaccination, should be “Freeze-dried BCG vaccine, Percutaneous”. The preparation should satisfy all the recommendations formulated below.

A.1.3 *International reference preparation/ reagents*

The 1st International Reference Preparation for BCG vaccine was established in 1965 and the 1st International Standard for PPD of *M. tuberculosis*, in 1951. Because of the age of these preparations, the need for replacements has been recognized, especially for the International Reference Preparation for BCG vaccine which is a live bacterial preparation. WHO has initiated the development of replacement for the BCG reference preparation. These have been presented to the ECBS in 2009 and 2010 as candidates for the 1st WHO Reference Reagents for BCG vaccines of sub-strain Danish 1331, Tokyo 172-1 and Russian BCG-I (23). These reference reagents cover the major proportion of BCG vaccine strains currently used in production. The establishment of sub-strain Moreau-RJ as the WHO Reference Reagent for BCG vaccine is currently in progress and scheduled to submit to the ECBS in 2012 for adoption. These preparations are intended as reference reagents if required for:

- periodical consistency monitoring of quantitative assays such as viability estimates (such as culturable particle count and modified ATP assays);
- residual virulence/ local reactogenicity assays and protection assays in animal models for nonclinical evaluation; and/ or

- as reference BCG sub-strains for identity tests using multiplex PCR as included in the collaborative study or in other molecular biology techniques.

The NIBSC-HPA, Potters Bar, UK distributes the WHO Reference Reagents for BCG vaccines.

A.1.4 *Terminology* *(alphabetical order)*

The definitions given below apply to the terms as used in these recommendations. They may have different meanings in other contexts.

Final bulk: The homogeneous finished liquid vaccine present in a single container from which the final containers are filled, either directly or through one or more intermediate containers derived from the initial single container.

Final lot: A number of sealed, final containers that are equivalent with respect to the risk of contamination during filling and, when it is performed, freeze-drying. A final lot should therefore have been filled from a single container and freeze-dried in one continuous working session.

In-house reference: A batch of vaccine prepared from the same BCG strain as the tested vaccine and used in parallel to the vaccine tested in:

- quantitative assays such as viability estimates (such as culturable particle count and modified ATP assays); and
- residual virulence assays.

Master seed lot: A bacterial suspension of a single sub-strain originated from the bacillus of Calmette and Guérin that has been processed as a single lot and is of uniform composition. A seed lot should be maintained in the freeze-dried form stored at -20°C or below (in the liquid form stored at -80°C or below) in order to maintain viability. In each manufacturing establishment, a master seed lot is that from which material is drawn for inoculating media for the preparation of working seed lots or single harvests.

Single harvests: The material obtained from one batch of cultures that have been inoculated with the working seed lot (or with the inoculum derived from it), harvested and processed together. Single harvests should be prepared from cultures originating from a seed lot by as few cultural passages as possible, and by not more than 12 passages from the master seed lot.

Working seed lot: A quantity of bacterial organisms of a single sub-strain derived from the master seed lot by growing the organisms and maintaining them in aliquots in the freeze-dried form stored at -20°C or below (in the liquid form stored at -80°C or below). The working seed lot should be

prepared from the master seed lot by as few cultural passages as possible, *e.g.* 3-6 passages from the master seed lot, having the same characteristics as the master seed lot and intended for inoculating media for the preparation of single harvests.

A.2 General manufacturing recommendations

The general manufacturing recommendations for manufacturing establishments contained in the *Good Manufacturing Practices for Pharmaceuticals Products: main principles* (24) and the *Good Manufacturing Practices for Biological Products* (25) should apply to establishments manufacturing BCG vaccine. Also, the compliance with current good manufacturing practices should apply with the addition of the following:

Details of standard operating procedures for the preparation and testing of BCG vaccines adopted by the manufacturer together with evidence of appropriate validation of each production step should be submitted for the approval of the NRA. As may be required, proposals for the modification of manufacturing and control methods should also be submitted for approval to the NRA before they are implemented.

The NRA should satisfy itself that adequate control of the manufacturing, shipping, and storage of the BCG vaccine has been achieved. NRAs may consider that a formal clinical lot-to-lot consistency study is not necessary if there are adequate and satisfactory data provided to support consistency of manufacture. However, several different lots of the product should be used in randomized studies and should elicit comparable immune responses in similar populations.

> The degree of consistency in producing satisfactory final lots is an important factor in judging the efficacy and safety of a particular manufacturer's product.

The data that should be considered in determining the consistency of production should include the results obtained with consecutive vaccine lots when tested as described in Part A, section 6, for example, the test for viability (Part A, section 6.7), and thermal stability test (Part A, section 6.8).

More than two consecutive vaccine lots should have been satisfactorily prepared before any vaccine from a given manufacturer, or resulting from a new method of manufacture, is released. In subsequent routine production, if a specified proportion of vaccine lots or a specified number of

consecutive vaccine lots fails to meet the requirements, the manufacture of BCG vaccine should be discontinued and not be resumed until a thorough investigation has been made and the cause or causes of the failures determined to the satisfaction of the NRA.

Conventionally, production of BCG vaccine should take place in dedicated area, completely separate from areas used for production of other medicines or vaccines, and using dedicated separate equipment. Such areas should be so situated and ventilated that the hazard of contamination is reduced to a minimum. No animals should be permitted in the vaccine production areas. Tests for the control of vaccine that require cultures to be made of contaminating microorganisms should be carried out in a completely separate area. Tests in which animals are used should also be carried out in a completely separate area.

For the purposes of these requirements, the processes of vaccine production that should take place in dedicated facilities are all operations up to and including the sealing of the vaccine in the final containers.

In some countries, the production of BCG vaccine - although isolated - is carried out in a building in which other work takes place. This should be done only after consultation with, and with the approval of, the NRA. If production takes place in part of a building, the work carried on in other parts of the building should be of such a nature that there is no possibility of cross-contamination to the BCG vaccine.

No cultures of microorganisms other than the BCG vaccine strain approved by the NRA for vaccine production should be introduced into the manufacturing areas. In particular, no strains of other mycobacterial species, whether pathogenic or not, should be permitted in the BCG vaccine production area.

BCG is susceptible to sunlight. Therefore, the procedures for the preparation of the vaccine should be so designed that all cultures and vaccines are protected from direct sunlight and ultraviolet light at all stages of manufacture, testing, and storage, until the vaccine is issued.

BCG vaccine should be produced by a staff consisting of healthy persons who do not work with other infectious agents; in particular, they should not work with virulent strains of *M. tuberculosis,* nor should they be exposed to a known risk of tuberculosis infection. Precautions should be taken also to ensure that no worker should be employed in the preparation of BCG vaccine unless he or

she has been shown by medical examination to be free from TB. The scope and nature of the medical examination should be at the discretion of the NRA, but it should include a radiological examination and should be repeated at intervals or when there is reason to suspect illness.

The frequency with which the radiological examination should be carried out is at the discretion of the NRA. It is advisable to keep radiation exposure to a minimum, but the examination should be of sufficient frequency to detect the appearance of early active TB. It is estimated that, if workers in BCG vaccine laboratories were given one or two conventional X-ray examinations of the chest each year, not using fluoroscopic methods, and if the best available techniques were employed to minimize the radiation dose, the doses received would be considerably lower than the maximum permissible doses for workers occupationally exposed to radiation that have been set by the International Commission on Radiological Protection (26, 27*).*

Should an examination reveal signs of TB or suspected TB in a worker, he or she should no longer be allowed to work in the production areas and the rest of the staff should be examined for possible TB infection. In addition, all cultures should be discarded and the production areas decontaminated. If it is confirmed that the worker has TB, all vaccine made while he or she was in the production areas should be discarded. In addition, distributed batches should be recalled.

Persons not normally employed in the production areas should be excluded from them unless, after a medical examination, including radiological examination, they are shown to be free from TB. In particular, persons working with mycobacteria other than the BCG seed strain should be excluded at all times.

Written descriptions of procedures for the preparation of BCG vaccine should be submitted for approval to the NRA. Proposals for modification should be submitted for approval to the NRA before their implementation.

A.3 Control of source materials

A.3.1 *Seed lot system*

The production of vaccine should be based on the seed lot system. A seed lot prepared from a strain approved by the NRA (see Part D, section 1.1) should be prepared under conditions satisfying the requirements of Part A, sections 2, 3 and 4.

The BCG vaccine strain used should be identified by historical records that include information on its origin and subsequent manipulation. It would be preferable for the master seed lot to have protection proven clinically through clinical studies with a batch derived from it by a production process that is representative of the commercial process; also it is recommended to use a batch derived from such a clinically 'validated' seed lot as in-house reference in the laboratory to help ensure consistency in production.

If a working seed lot is being used, the total number of passages for a single production harvest should not exceed 12 including the passages necessary for preparing the working seed lot.

Clinically relevant antimicrobial sensitivity testing should be carried out as a part of the ongoing characterization of BCG sub-strains. It would be appropriate to test this property at the level of both master and working seed lots for licensing purposes and to monitor this in final lot.

A.3.2 *Tests on seed lot*

When a new working seed lot is established, a suitable test for delayed hypersensitivity in guinea-pigs is carried out; the vaccine is show to be not significantly different in activity from the in-house reference.

A.3.2.1. *Identity test*

The bacteria in the master and working seed lots are identified as *M. bovis* BCG using microbiological techniques, for example morphological appearance of the bacilli in stained smears and by the characteristic appearance of the colonies grown on solid media. Molecular biology techniques, for example PCR test can supplement to identify the specific sub-strain of BCG. The techniques will also ensure genetic consistency in production, from master seed through working seed and to final product (4).

A.3.2.2. *Test for bacterial and fungal contamination*

Each master and working seed lot should be tested for bacterial and fungal contamination by appropriate tests as specified in Part A, section 5.2 (28) of the *General Requirements for the Sterility of Biological Substances,* or by the validated methods approved by the NRA.

A.3.2.3 *Test for absence of virulent mycobacteria*

The test for absence of virulent mycobacteria, described in Part A, section 4.2.3, should be made in at least ten healthy guinea-pigs injected with a quantity of vaccine not less than 50 single human doses and should be observed for at least 6 weeks. If none of the animals shows signs of progressive TB and at least 90% survive (i.e. should 1 out of 10 animals dies) the observation period, the seed lot should be considered to be free from virulent mycobacteria .

Should more than 10% of the guinea-pigs die (i.e. should 2 out of 10 animals die) during the observation period and freedom from progressive TB disease is verified, the test should be repeated on at least 10 more guinea-pigs . On the second occasion, the seed lot passes the test if not more than 10% animal die (i.e. should 1 of 10 animals dies) during the observation period and autopsy does not reveal any sign of TB .

A.3.2.4. *Test for excessive dermal reactivity*

Use 6 healthy guinea-pigs, each weighing not less than 250g and having received no treatment likely to interfere with the test. Inject intradermally into each guinea-pig, according to a randomized plan, 0.1 ml of the reconstituted vaccine and of vaccine dilutions 1:10 and 1:100. The same dilutions of the appropriate international reference reagent or in-house reference should be injected into the same guinea-pigs at randomly selected sites. Observe the lesions formed at the sites of injection for at least 4 weeks. The vaccine complies with the test if the reactions it produces are not markedly different from that produced by the appropriate international reference reagent or in-house reference.

A.3.3 *Production culture medium*

The production culture medium should contain no substances known to cause toxic or allergic reactions in humans. The use of material originated from animals should be discouraged. However, if constituents derived from animal origin are necessary, approval of the NRA should be sought and the materials should comply with current Transmissible Spongiform Encephalopathies (TSE) policy (29, 30, 31, 32, 33, 34). A risk assessment for TSE would need to be included for the materials of culture medium. The revised WHO Guidelines on TSE in relation to biological and pharmaceutical products (29) provide guidance on risk assessments for master and working seeds and should be consulted. Substances used in that medium should meet such specifications as the NRA may prescribe.

A.4 Control of vaccine production

A.4.1 *Control of single harvests*

All cultures should be examined visually, and any that have grown in an uncharacteristic manner should not be used for vaccine production.

A.4.2 *Control of final bulk*

A.4.2.1 *Final bulk*

The final bulk should be prepared from a single harvest or by pooling a number of single harvests.

A.4.2.2 *Test for bacterial and fungal contamination*

The final bulk should be tested for bacterial and fungal contamination by appropriate tests as specified in Part A, section 5.2 (28) of the *General Requirements for the Sterility of Biological Substances,* or by the validated methods approved by the NRA. No vaccine lot should be passed for use unless the final bulk has been shown to be free from such contamination.

A.4.2.3 *Test for absence of virulent mycobacteria*

The test for absence of virulent mycobacteria should be carried out on each final bulk or final lot.

At least 6 healthy guinea-pigs, all of the same sex, each weighing 250 - 400 g are used. They have not received any treatment or diet, such as antibiotics, likely to interfere with the test. A sample of the final bulk intended for this test should be stored at 4°C for not more than 72 hours after harvest.

A dose of BCG organisms corresponding to at least 50 single human doses of vaccine intended for intradermal injection should be injected into each guinea-pig by the subcutaneous or intramuscular route.[1] The guinea-pigs should be observed for at least 6 weeks. If, during that time, they remain healthy, gain weight, show no signs of progressive TB and not more than one die, the final bulk should be considered to be free from virulent mycobacteria .

[1] When a more concentrated vaccine, intended for administration by the percutaneous route, is tested, a dilution factor approved by the NRA should be applied so that the mass of BCG injected corresponds to at least 50 human doses of intradermal vaccine.

At the end of the observation period, the animals should be sacrificed and examined post-mortem for macroscopic evidence of progressive TB disease; similarly, any animals that die before the end of the observation period should be subjected to a post-mortem examination.

Should one-third of the guinea-pigs die (i.e. should 2 out of 6 animals die) during the observation period (and freedom from progressive TB disease is verified), the test should be repeated on at least 6 more guinea-pigs.

On the second occasion, the vaccine lot passes the test if not more than one animal dies during the observation period and autopsy does not reveal any sign of TB.

Should a vaccine lot fail to satisfy the requirements of this test because animals die from causes other than TB, the procedure to be followed by the manufacturer should be determined with the approval of the NRA.

If signs of TB disease are seen, the vaccine lot should be rejected, all subsequent vaccine lots should be withheld, and all current vaccine stocks should be held pending further investigation. The manufacture of BCG vaccine should be discontinued and it should not be resumed until a thorough investigation has been made and the cause or causes of the failure determined and appropriate actions have been taken. Production should be allowed to resume only upon the approval of the NRA.

A.4.2.4 *Test for bacterial concentration*

The bacterial concentration of the final bulk should be estimated by a validated method approved by the NRA and should have a value within a range approved by the NRA (see Part D, section 1.2).

Based on manufacturers' experience, the opacity method is the method of choice, the International Reference Preparation of Opacity,[1] or an equivalent reference preparation approved by the NRA, may be employed in comparative tests.

A.4.2.5 *Test for number of culturable particles*

The number of culturable particles on a solid medium of each final bulk should be determined by an appropriate method approved by the NRA. Alternatively, a bioluminescense or other biochemical method can be used (35, 36), provided that the method is properly validated against the culturable particle test, for the production step in question. If properly validated, such tests can be used as

[1]The International Reference Preparation of Opacity is in the custody of the National Institute for Biological Standards and Control, Health Protection Agency, Potters Bar, Hertfordshire, England, which supplies samples on request.

equivalent methods. Regular calibration with the reference method as agreed with NRA would be relevant.

The medium used in this test should be such that the number of culturable particles may be determined at an optimal time point (usually 3-5 weeks) after the medium has been inoculated with dilutions of the vaccine.

There are various methods of determining the number of culturable particles in BCG vaccine, and it is essential that only one culture method be used for all the vaccine lots produced by a manufacturer (5). It is also desirable for assay validation that the test be carried out in parallel with the appropriate international reference reagent or in-house reference, e.g. the same vaccine that has been used in clinical trials and assured safety (including immunogenicity) and efficacy.

A.4.2.6 *Substances added to the final bulk*

Substances used in preparing the final bulk should meet such specifications as the NRA may prescribe. In particular, the NRA should approve the source(s) of any animal-derived raw materials that should comply with the guidelines on tissue infectivity distribution of TSEs (30).

Substances added to improve the efficiency of the freeze-drying process or to aid the stability of the freeze-dried product should be sterile and of high and consistent quality, and should be used at suitable concentrations in the vaccine.

A.5 Filling and containers

The general requirements concerning filling and containers given in *Good Manufacturing Practices for Biological Products* (25) should apply to vaccine filled in the final form.

The containers should be in a form that renders the process of reconstitution as simple as possible. Their packaging should be such that the reconstituted vaccine is protected from direct sunlight.

A.6 Control tests on final lot

Tests on the final lot should be performed after reconstitution, except for appearance and residual moisture tests. The fluid supplied or recommended for reconstitution should be used, unless such fluid would interfere with any of the tests, in which case some other suitable fluid should be used. The vaccine should be reconstituted to the concentration at which it is to be used for injection into humans; an exception may be made in the case of the test for absence of virulent mycobacteria (Part A, section 6.4.1), when a higher concentration of reconstituted vaccine may be necessary.

A.6.1 *Inspection of final containers*

Every container in each final lot should be inspected visually, and those showing abnormalities should be discarded.

The appearance of the freeze-dried vaccine and the reconstituted vaccine should be described with respect to its form and colour. If reconstitution with the product diluent does not allow for the detection of particulates, an alternative diluent may be used.

A.6.2 *Identity test*

An identity test should be performed on samples of the vaccine from each final lot. The identity test for final lots should be used to identify the product as BCG as approved by NRA. The identity of each final lot of vaccine should be verified by the morphological appearance of the bacilli in stained smears and by the characteristic appearance of the colonies grown on solid media. Preferably a validated nucleic acid amplification technique (such as PCR) should be used and the morphological technique.

A.6.3 *Test for bacterial and fungal contamination*

Samples from each final lot should be tested for bacterial and fungal contamination by appropriate tests as specified in Part A, section 5.2 (28) of the *General Requirements for the Sterility of Biological Substances,* or by the validated methods approved by the NRA.

A.6.4 *Safety tests*

A.6.4.1 *Test for absence of virulent mycobacteria*

Provided the test for virulent mycobacteria has been carried out with satisfactory results on the final bulk vaccine, it may be omitted on the final lot.

If the test for the absence of virulent mycobacteria, applied to the final bulk, is unsatisfactory (and freedom from progressive TB disease is verified), it should be repeated with a sample of a final lot (see Part A, section 4.2.3).

A.6.4.2 *Test for excessive dermal reactivity*

Provided the test has been carried out with satisfactory results on the working seed lot and on 5 consecutive final lots produced from it, the test may be omitted on the final lot.

A.6.5 *Test for bacterial concentration*

The total bacterial content of the reconstituted vaccine should be estimated for each vaccine lot by a validated method approved by the NRA, and should have a value within a range approved by the NRA (see Part D. section 1.2).

The estimation of tota1 bacterial content may be made either directly, by determining the dry weight of organisms, or indirectly, by an opacity method that has been calibrated in relation to the dry weight of the organisms.

It is desirable that one method of estimation should be adhered to for all the vaccine lots produced by a manufacturer.

A.6.6 *Test for residual moisture*

The average moisture content of a freeze-dried vaccine should be determined by a validated method accepted by the NRA. Values should be within limits of the preparations shown to be adequately stable in the stability studies of the vaccine.

A.6.7 *Tests for viability*

A.6.7.1 *Test for number of culturable particles*

The number of culturable particles of each final lot should be determined by an appropriate method approved by the NRA (see Part A, section 4.2.5). The viable count should have a value within a range approved by the NRA that should not be wider than a 4-fold difference between the lower and upper levels of the specification for numbers of culturable particles (see Part D, section 1.2). By comparison with the results of the test for number of culturable particles carried out on final bulk, as described in Part A. section 4.2.5, the percentage survival on freeze-drying may be calculated and this value should be not less than one approved by the NRA. The appropriate international reference reagent or in-house reference should be used for every test in order to validate the assay.

The purpose of including the appropriate international reference reagent or in-house reference is to have a check on the quality and consistency of the culture medium and the accuracy of the technique used for the determination of the number of culturable particles. It is not intended to adjust the count of the vaccine by comparison with the reference preparation.

The survival rate after freeze-drying is usually not less than 20%.

A.6.7.2 *Rapid test for viability*

As an alternative to the colony counting method, a bioluminescense or other biochemical method can be used, provided that the method is properly validated against the culturable particle test, for the production step in question. If properly validated, such tests may be considered by the NRA to replace the culturable particle test.

The bioluminescence reaction occurring in fireflies depends upon the presence of adenosine triphosphate (ATP), luciferin luciferase, oxygen, and magnesium ions. This reaction can be reproduced *in vitro* by mixing these components. If all components except ATP are present in excess, the amount of light emitted is proportional to the amount of ATP coming from the vaccine.

Since ATP is present in all living cells and is immediately destroyed when the cell dies, ATP is a reliable marker for living cells.

Studies, have shown that if properly validated, measurement of ATP using the bioluminescence reaction can be used to estimate the viable count of freeze-dried BCG vaccine within 1- 2 days, as accurately as other, more time-consuming methods, once the mean content of ATP per culturable particle has been estimated for a given vaccine production.

A.6.8 *Thermal stability test*

The thermal stability test is as part of characterization and consistency demonstration of the vaccine production. This requirement of this test should be at discretion of NRA and if required, each final lot should be tested for thermal stability by a validated method approved by the NRA. If the production consistency is demonstrated, this test may be omitted on the final lot and subjected to NRA approval (6).

If performed, the test should involve the determination of the number of culturable particles before and after the samples have been held at appropriate temperatures and for appropriate periods.

For example, the thermal stability test may be carried out by taking samples of the vaccine and incubating them at 37°C for 28 days.

The percentage decrease in the number of culturable particles is then compared with that of samples of the same vaccine lot stored at 2° - 8°C. The number of culturable particles in the vaccine after heating should be not less than 20% of that stored at 2° - 8°C (37). The absolute value should be approved by the NRA. The viability test should also be performed with the appropriate international reference reagent or in-house reference for checking validity of the assay. One method of

determining the number of culturable particles should be adhered to, as suggested in Part A, section 4.2.5.

The purpose of including the appropriate international reference reagent or in-house reference is to have a check on the quality and consistency of the medium used for the determination of the number of culturable particles. It is not intended to adjust the count of the vaccine by comparison with the reference preparation.

All manufacturers should keep their product for the approved storage period and should determine the number of culturable particles from time to time to demonstrate that the number is being maintained at an adequate level.

In some countries, the thermal stability test is carried out only after the vaccine has been stored for 3-4 weeks after freeze-drying, since it is considered that the degree of stability during the first 3 weeks may not be related to the long-term stability of the product.

As a guide to stability, some manufacturers of freeze-dried BCG vaccine determine the residual moisture content of the final vaccine, since failure to achieve a certain degree of desiccation results in an unstable product. However, such a test cannot be regarded as an alternative to tests involving the determination of the number of culturable particles.

A.7 Records

The recommendations in Section 8 of *Good Manufacturing Practices for Biological Products* should apply (25)

Written records should be kept of all seed lots, all cultures intended for vaccine production, all single harvests, all final bulk vaccines, and all vaccine in the final containers produced by the manufacturing establishments, including all tests irrespective of their results.

The records should be of a type approved by the NRA. An example of a suitable protocol is given in Appendix 2.

A.8 Retained samples

The recommendations in Section 9.5 of *Good Manufacturing Practices for Biological Products* should apply (25).

It is desirable that samples should be retained for at least one year after the expiry date for the final lot.

A.9 Labeling

The recommendations in Section 7 of *Good Manufacturing Practices for Biological Products* (25) should apply including the following.

The label, and/ or the packaging insert in some countries, printed on or affixed to each container should show the volume and nature of the reconstituting fluid. Also, this label, or the label on the carton enclosing several final containers, or the leaflet accompanying the containers, should contain the following additional information:

- the fact that the vaccine fulfils the requirements of this document;
- instructions for use of the vaccine and information concerning contraindications and the reactions that may follow vaccination;
- the conditions recommended during storage and transport, with information on the reduced stability of the vaccine if exposed to temperatures higher than that stated on the label;
- warnings that the vaccine should be protected from direct sunlight;
- a statement that, after a final container of freeze-dried BCG has been reconstituted, the vaccine should be kept on ice or otherwise refrigerated until used, should be used as soon as possible, and that any reconstituted container remaining at the end of the immunization session (maximum six hours) should be discarded (38), and
- information on clinically relevant antimicrobial sensitivity.

The label for the reconstituting fluid should state 'Reconstituting fluid for BCG vaccine Proprietary name'.

A.10 Distribution and transport

The recommendations given in Section 8 of *Good Manufacturing Practices for Biological Products* (25) should apply. Also, the document for Safe Vaccine Handling, Cold Chain and Immunizations (39) should apply. Further guidance is provided in the *WHO Model Guidance for the Storage and Transport of Time and Temperature–sensitive Pharmaceutical Products* (40).

Diluent used in reconstitution should be shipped and distributed together with the vaccine in immediate container, i.e. vial or ampoules (41). This ensures that

the correct diluent will be used for the vaccine. The freeze-dried vaccine is not damaged by freezing and can be frozen and thawed. However, repeated freeze-thawing is not recommended. The diluent should never be frozen.

A.11 Stability, storage and expiry date

A.11.1 *Stability testing*

Adequate stability studies form an essential part of vaccine development**.** Current guidance on evaluation of vaccine stability is provided in the recommendations given in WHO *guidelines on stability evaluation of vaccines* should be applied (42). Stability testing should be performed at different stages of production if stored for a given time period, namely as appropriate on single harvests or pool of single harvests, final bulk, final lot. In addition, such studies should be undertaken on reconstituted vaccine. Stability-indicating parameters should be defined or selected appropriately according to the stage of production. It is advisable to assign a storage period to all in-process materials during vaccine production, in particular intermediates such as single harvests and final bulk; and a shelf-life period to the final lots.

BCG vaccines require special precautions to ensure sufficient stability. In this connection the most important measures are lyophilization, the use of an effective stabilizer, and proper sealing of vaccine containers.

Historically the use of ampoules sealed under vacuum was the most common practice for increasing stability. However, vacuum-sealing is difficult compared to sealing in the presence of inert gas. There were no significant differences between BCG vaccines sealed under vacuum and under nitrogen or carbon dioxide at either 4° or 37°C (19). Manufacturers now prepare BCG vaccines in vials/ ampoules, and under well-validated conditions, the product is adequately stable.

A.11.2 *Storage conditions*

The Guideline for Establishing or Improving Primary and Intermediate Vaccine Stores (41) should apply.

Storage conditions should be based on stability studies and approved by the NRA. Before being distributed by the manufacturing establishment, or before being issued from a depot for the storage of vaccine, all vaccines in their final containers should be stored constantly at 2°-8°C (37, 44) and vaccine diluents should be stored as recommended by manufacturer. Freeze-dried BCG vaccines,

regardless of their sub-strain, are sensitive to ultraviolet and fluorescent light. They should be protected from direct sunlight (37).

BCG vaccines are sensitive to light as well as to heat. Normally, these vaccines are supplied in vials/ ampoules made from dark brown glass, which gives them some protection against light damage, but care should still be taken to keep them covered and protected from strong light at all times (44).

Freeze-dried BCG vaccines may be kept frozen at -15°C to -25°C if cold chain space permits, but this is neither essential nor recommended (37).

Precautions should also be taken to maintain the vaccine, during transport and up to the time of use, at the temperature and under the storage conditions recommended by the manufacturer.

A.11.3 ***Expiry date***

The expiry date should be approved by the NRA and based on the stability of the final product as well as the results of the stability tests referred to in section 11.1. It is established for each batch by adding the shelf-life period to the date of manufacture. Most freeze-dried BCG vaccines are stable at temperatures of 2°-8°C for at least two years (37) from the date of manufacture. The storage of final product at -20°C to extend the shelf-life should be validated.

Freeze-dried BCG vaccines become much more heat sensitive after they have been reconstituted with diluent. Reconstituted BCG vaccine is very unstable and at risk of contamination (37, 39, 44). Once reconstituted, BCG vaccine should be stored on ice or at 2°-8°C and use within 6 hours (4, 41).

Part B. Preclinical evaluation of BCG vaccines

Details on the design, conduct, analysis and evaluation of preclinical studies are available in *WHO Guidelines for Nonclinical Evaluation of Vaccines* (45).

Preclinical testing of a new strain (*i.e.* derived by selection from existing BCG strains in Appendix 1) or for a new manufacturer of a BCG vaccine is a prerequisite for initiation of clinical studies in humans, and includes immunogenicity, protection studies (proof of concept) and safety testing in animals. The vaccine lots used in preclinical studies should be adequately representative of the formulation intended for clinical investigation and, ideally, should be the same current Good Manufacturing Practice (cGMP) manufactured lots used in clinical studies. If this is not feasible, then the lots used clinically should be comparable to those used in the preclinical studies with respect to potency, stability and other characteristics of quality, often the technical manufacturing consistency lots may be used for these purposes.

New manufacturers of BCG vaccine for human use will need to refer to the range of preclinical safety and characterisation tests that are recommended for existing, licensed BCG vaccines. Although there is currently no requirement for additional preclinical testing beyond that already described for licensed BCG vaccines, the development of new variants of BCG, the potential for new fermentation technologies and the possibility of novel live vaccines against TB have shown that additional preclinical studies beyond that required of licensed BCG vaccine can be helpful in demonstrating that a new BCG product has satisfactory preclinical efficacy, safety and stability.

> Guideline example on protective potency testing: Hartley Guinea-pigs are used for potency testing. Guinea-pigs are vaccinated with a small amount of BCG (~10^3 CFU). Eight weeks after the vaccination, guinea-pigs are challenged with virulent *M. tuberculosis* H37Rv (ATCC 27294) by the pulmonary route with a low dose (10 – 15 CFU) per animal. Five weeks after the infection, guinea pigs are euthanized, the spleen and the lung lobes are removed. Then these organs are homogenized separately. Appropriate dilutions are inoculated onto duplicate solid medium and incubated at 37°C for 3 weeks. The number of *M. tuberculosis* H37Rv colonies is counted, and expressed as mean $\log_{10}$ CFU per tissue. The CFU results are compared between the vaccinated and non-vaccinated groups (46).

If there are two pharmacologically relevant species for the clinical candidate (one rodent and one non-rodent), then both species should be used for short-term (up to 1 month duration) toxicology studies. If the toxicological findings from these studies are similar in both species, then longer-term

866 studies in one species are usually considered sufficient; the rodent species should be considered
867 unless there is a rationale for using non-rodents. Studies in two nonrodent species are not
868 appropriate. Other *in vivo* studies should address both potency (such as tuberculin sensitivity and
869 immunological tests) and safety (such as tests for excessive dermal reactivity and absence of
870 virulent mycobacteria) issues of the classical BCG vaccines.
871
872 It may be of benefit for new BCG vaccine developers to consider the points raised in the recent
873 meetings establishing recommendations for new live vaccines against TB (47, 48).

Part C. Clinical evaluation of BCG vaccines

Clinical trials should adhere to the principles described in the *WHO Guidelines for Good Clinical Practice (GCP) for Trials on Pharmaceutical Products* (49) and the general principles described in the *WHO Guidelines on Clinical Evaluation of Vaccines: Regulatory Expectations* (50). All clinical trials should be approved by the relevant NRAs and local Ethics Committees. Continued licence of BCG vaccines should be viewed in the light of on-going post-marketing data on the safety, immunogenicity and effectiveness of BCG vaccines in the target population.

The section considers the provision of clinical data required *a)* when a new candidate "classical" BCG vaccine derived from (the same master seed of) one of the strains recognized (see Appendix 1) is developed; *b)* when there have been major changes to the manufacturing process of an established vaccine, including preparation of new master seed lot of an established strain; *c)* when technology transfer of existing vaccine is planned to a new manufacturer; and *d)* when revalidation of existing vaccines used in national immunization program is considered.

Vaccines manufactured using a "new strain (*i.e.* derived by selection from existing BCG strains in Appendix 1)" should require a full clinical development program that provides evidence of safety, efficacy, and the reactogenicity profile in all age target age groups.

Other vaccines against *M tuberculosis* derived from *M. bovis* or other mycobacterial strains cannot be considered as “BCG” and would require a full clinical development program and are not included here.

C.1 General considerations

C.1.1 *Comparative or Placebo-controlled clinical trials*

It would not be considered ethical to conduct a placebo-controlled trial of protective efficacy of a BCG vaccine in a TB endemic area, particularly in infants. A comparative trial with a licensed, or internationally accepted (WHO pre-qualified) BCG vaccine could be accepted.

C.1.2 *Value of PPD response*

It is recognized that the response to PPD is not an indicator of a protective immune response. Nonetheless this has been used for over 50 years to indicate a cellular immune response to an infection with *M. tuberculosis* or as evidence of "successful" BCG vaccination. At best a PPD reaction is an indicator of exposure to antigens of TB, and the generation of a cellular immune response. Thus, it can be used in a PPD naïve population as an indicator of an immune response to the BCG vaccine (51). Other immunological measures may be more closely related to *M. tuberculosis* infection or vaccination, but currently none has been agreed as a correlate of protection from infection or disease.

C.1.3 *BCG in HIV-infected infants*

A very important safety consideration with regard to vaccination policy is establishing, during clinical trials, the potential for disseminated BCG disease in immunocompromised children that may be more pronounced. The use of BCG vaccines at birth should follow the recommendations from WHO Strategic Advisory Group of Experts (SAGE) on immunization and position papers (13, 14). These consider the policies for immunization exclusion for infants known to be infected with HIV, infants symptomatic for HIV infection, and those infants, born to mothers known to be HIV infected, and who may be infected.

C.1.4 *Post-vaccination reactions and complications*

Vaccines intended for intradermal or percutaneous injection should be given strictly intradermally or percutaneously, and vaccinators should be trained accordingly. Incorrect vaccination technique can result in adverse reactions, including discharging ulcers, abscesses and keloid scars.

Current BCG vaccines have a known reactogenicity profile after intradermal inoculation (52). Local reaction at the vaccination site is normal after a BCG vaccination. It may take the form of a nodule that, in many cases, will break down and suppurate. The reaction developing at the vaccination site usually subsides within 2 - 5 months and in practically all children leaves a superficial scar of 2 - 10 mm in diameter. The nodule may persist and ulcerate. Swelling of regional lymph nodes may also be seen, and this may be regarded as a normal reaction, but the size should be limited.

Keloid and lupoid reactions may occur at the site of the vaccination. Children with such reactions should not be revaccinated. Inadvertent subcutaneous injections produce abscess formations and

may lead to ugly retracted scars. Among the major complications, suppurative lymphadenitis has been observed. In the case of certain vaccines, it has been revealed that there is a strong correlation between the incidence of these complications in newborns and the number of culturable particles in the vaccine.

Thus, a reduction of the dose for the newborn may reduce these complications to acceptable levels. It is recommended that the dose for newborns or infants should be one-half to one-quarter of that for teenage children or adults. The concentration of the vaccine should be shown to be effective and tolerated in the age groups for which the vaccine is intended (53).

The NRA should issue guidelines for the treatment of complications.

C.2 Special considerations

C.2.1 *New "classical" BCG vaccines*

This section is limited to the clinical development of new "classical" BCG vaccines manufactured following these recommendations and using strains of BCG that are derived from (the same master seed of) one of the strains recognized in Appendix 1.

The use of comparative studies with a licensed BCG vaccine can provide evidence of the similarity of safety and immune responses to a new classical BCG vaccine product.

The target population for the vaccine would be newborns or infants according to the current recommendations for use of BCG vaccines.

The preclinical expectations for a new classical BCG vaccine are outlined in Part B.

> For such a new classical BCG vaccine, these preclinical studies should be conducted in comparison to an existing licensed BCG vaccine, preferably derived from the same BCG sub-strain. It would be expected that the results of preclinical studies would be similar for the new vaccine product and for the comparator.

The clinical development program should ideally be designed to show the safety and protective efficacy for the vaccine. However, for such a new classical BCG vaccine product, comparative studies with an existing licensed BCG vaccine, using immunological responses as a marker for efficacy, may be acceptable to the responsible NRA.

Comparable PPD response (proportion of PPD converters, intensity of response) may be acceptable.

Clinical studies should provide evidence of safety in all the potential target populations, including those with a high incidence of diseases that may affect the safety or efficacy of the new vaccine product.

Phase-I/II: Safety and reactogenicity in healthy adults (comparative)

End points

Safety and reactogenicity – can include healthy HIV-infected adults

Immune responses – non-inferior PPD response and may include other immunological markers.

These studies are difficult to interpret as adults will most likely have received BCG vaccination at birth. Dose-finding studies may be considered unnecessary for these vaccines. The safety in HIV-infected individuals and infants needs to be considered.

Dose-finding and age-de-escalation can be included in these studies but review by a suitable Independent Safety Committee at each step should be considered.

Phase-III: Safety and reactogenicity in infants (comparative)

End points

Safety and reactogenicity

Non-inferior PPD immune response

Post-marketing risk management:

As it may not be practically possible to evaluate protective efficacy for a new classical BCG vaccine, the responsible NRA in the country of manufacture should require post-marketing surveillance activities for safety and effectiveness in a suitable environment. Sentinel surveillance sites in an endemic country may be considered.

C.2.2 *Revalidation of existing vaccines within national immunization program*

The responsible NRA of a country of manufacture may require a demonstration that adequate control of BCG vaccine has been achieved, by arranging for studies in children to be made at regular intervals on some of the final lots prepared.

Such studies on immunological responses to *M. tuberculosis* antigens should be made, including sensitivity to tuberculin. In at least 100 tuberculin-negative persons per year, and records should be obtained of the degree of sensitivity to tuberculin induced (distribution of tuberculin reactions by size)[1] with a defined dose of tuberculin,[2] local skin lesions (nature and size of reaction at injection site), and the occurrence of untoward vaccination reactions. It is desirable that such tests should be performed in parallel on two or more vaccine lots in the same population group, one of the vaccine lots being preferably a reference vaccine.

In relation to the tuberculin sensitivity test, different practices have been adopted according to the country situation. In the United State of America, Germany and Republic of Korea, routine demonstration of BCG-induced tuberculin conversion in humans is currently not required. This test is used in the UK as a diagnostic tool for TB disease in high risk children before BCG vaccination; and tuberculin-positive children are not vaccinated.

The frequency of testing of batches will depend on the number of batches of vaccine produced, but, in any case, at least one batch each year should be tested. The age groups of children in whom the vaccine is tested should be the same as those in which the vaccine will be eventually used.

If a batch of vaccine is to be exported, it should be ascertained in which age group it will be used in the importing country; the vaccine should be then tested accordingly.

C.3 Post-marketing surveillance

The responsible NRA in the country of manufacture may require periodic safety update reports and periodic revalidation of the BCG safety, and immunogenicity.

C.3.1 *BCG vaccine used in a national immunization program*

[1] In some countries, the proportion of cases showing a negative reaction to tuberculin before BCG vaccination, but giving a positive result after vaccination, is called the "tuberculin conversion rate". Unless positive and negative reactions are carefully defined, however, such a rate may not include certain cases in which a weak reaction to tuberculin is changed after BCG vaccination into a strong reaction.

[2] An intradermal test with a dose of tuberculin equivalent to 5 IU of tuberculin PPD is suitable. A description of an appropriate method and a design for a study to assess BCG vaccines in man are available on application to Chief, Tuberculosis and Respiratory Infections, World Health Organization, 1211 Geneva 27, Switzerland.

As in all immunization programmes, the adverse events following immunization with BCG vaccines should be monitored.

For BCG vaccines the following are important:

- All injection site abscesses;
- All cases of BCG lymphadenitis;
- All deaths that are thought by health workers, or the public, to be related to immunization;
- All cases requiring hospitalization that are thought by health workers, or the public, to be related to immunization; and
- Other severe or unusual medical incidents that are thought by health workers, or the public, to be related to immunization.

Appropriate training of health care workers is important as some medical incidents can be related to immunization even if they have a delayed onset (54).

C.3.2 *WHO pre-qualified BCG vaccines*

Pre-qualified vaccines may be used in a wide range of countries world-wide. Periodic safety update reports supplied to WHO should include specific analysis of countries where the vaccine has been used.

Part D. Recommendations for national regulatory authorities

D.1 General

The general recommendations for NRAs provided in the *Guidelines for National Authorities on Quality Assurance for Biological Products* should apply (55). These specify that no new biological substance should be released until consistency of manufacturing and quality as demonstrated by a consistent release of batches has been established. The detailed production and control procedures as well as any significant change in them that may affect the quality, safety or efficacy of BCG vaccine should be discussed with and approved by the NRA. For control purposes, the NRA should obtain the WHO Reference Reagents as comparators for potency-related testing and, where necessary, establish national working reference preparation(s) calibrated against the international reference. In addition, the NRA should provide a reference vaccine or approve one used by a manufacturer, and should give directions concerning the use of the reference vaccine in specified tests. The NRA should also give directions to manufacturers concerning the BCG sub-strain to be used in vaccine production, the total content of bacteria, the number of culturable particles, and the stability required of the vaccine, and should specify the requirements to be fulfilled by the manufacturer in accordance with the provisions of Part A of this document, including those for consistency of quality in respect of the points referred to in Part A, section 2.

D.1.1 *BCG vaccine strain*

The sub-strain of BCG (maintained in the form of a seed lot) used in the production of vaccine should be derived from the original strain maintained by Calmette and Guérin and should be identified by historical records that include information on its origin and subsequent manipulation. On the basis of cultures and biochemical and animal tests, the BCG seed lot should show characteristics that conform to those of BCG and generally differ from those of other mycobacteria. The identity test should be supplemented by molecular biology techniques to identify the specific BCG sub-strain used. The seed lot should show consistency in the morphological appearance of colonies and genetic stability on serial subculture. It should also have been shown to yield vaccines that, upon administration by intradermal injection to children and adults, induce relevant immunological responses to *M. tuberculosis* antigens including sensitivity to tuberculin, and with a

low frequency of untoward effects. In addition, the seed lot should have been shown to give adequate protection against TB in experimental animals in tests for protective potency.

D.1.2 *Concentration of BCG vaccine*

The concentration of BCG vaccine varies with different vaccine products and is dependent on a number of factors, such as the sub-strain of BCG used and the method of manufacture. It is therefore essential, for each manufacturer as well as for each different method of manufacture, for the optimum potency of vaccine to be ascertained by trials in tuberculin-negative subjects (newborns, older children, and adults) to determine the response to vaccination in respect of the induction of relevant immunological responses to *M. tuberculosis* antigens including sensitivity to tuberculin, the production of acceptable local skin lesions, and the occurrence of a low frequency of untoward reactions. As a result of such trials, the NRA should give directions to the manufacturer concerning the total bacterial content and the number of culturable particles required for the vaccine.

If a manufacturer changes its procedure of preparing BCG vaccine, and if the NRA considers that the change might affect the final product, it may be necessary to conduct further clinical trials in order to determine the optimum content of BCG organisms in the new product.

D.2 Release and certification[1]

A vaccine lot should be released only if it fulfils the national requirements and/or Part A of these Recommendations. Before any vaccine lot is released from a manufacturing establishment, the recommendations for consistency of production provided in *Guidelines for national authorities on quality assurance for biological products* (55) should be met. Also, the general recommendations for NRAs provided in the *Guidelines for Independent Lot Release of Vaccines by Regulatory Authorities*, which has been prepared, should be followed (56). A protocol based on the model given in Appendix 2, signed by the responsible official of the manufacturing establishment, should be prepared and submitted to the NRA in support of a request for release of vaccine for use.

A statement signed by the appropriate official of the NRA (or authority as appropriate) should be provided if requested by a manufacturing establishment and should certify whether or not the lot of vaccine in question meets all national requirements, as well as Part A of these recommendations. The certificate should also state the date of manufacture, the lot number, the number under which

[1] Where there is no NRA, a manufacturer may request advice and help from: Chief, Biologicals, World Health Organization, 1211 Geneva 27, Switzerland

the lot was released, and the number appearing on the labels of the containers. In addition, the date of the last satisfactory potency test as well as the expiry date assigned on the basis of shelf-life should be stated. A copy of the official national release document should be attached. The certificate should be based on the model given in Appendix 3. The purpose of the certificate is to facilitate the exchange of vaccines between countries.

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Dr Murielle Andre, Agence Française de Sécurité Sanitaire des Produits de Santé, Lyon, France; Dr Lewellys F. Barker, Aeras Global Tuberculosis Vaccine Foundation, Rockville, MD, USA; Dr Arun Bhardwaj, Central Research Institute, Kasuli, Himachal Pradesh, India; Dr Michael Brennan, Aeras Global TB Vaccine Foundation, Rockville, MD, USA; Dr Iin Susanti Budiharto, Bio Farma, Bandung, Indonesia; Dr Luiz Roberto Castello-Branco, Fundacao Ataulpho de Paiva, Brazilian League Against Tuberculosis, Rio de Janeiro, Brasil; Dr Miliana Chouchkova, National Center of Infectious and Parasitic Diseases, Sofia, Bulgaria; Dr Hyungok Chun, Korea Food and Drug Administration, Seoul, Republic of Korea; Dr Mike Corbel, NIBSC, Potters Bar, UK; Dr Bernd Eisele, Vakzine Projeckt Management GmbH, Hannover, Germany; Dr Sunil Gairola, Serum Institute of India, Hadapsar, India; Dr Leander Grode, Vakzine Projeckt Management GmbH, Hannover, Germany; Dr Kaare Haslov, Statens Serum Institut, Copenhagen, Denmark; Dr Mei Mei Ho, NIBSC, Potters Bar, UK; Dr Peter Hubrechts, Statens Serum Institut, Copenhagen, Denmark; Dr Greg Hussey, South African TB Vaccine Initiative, Cape Town, South Africa; Professor Diana Levi, Tarassevich State Research Institute for Standardization and Control of Medical Biological Preparations, Moscow, Russian Federation; Dr Kevin Markey, NIBSC, Potters Bar, UK; Dr Carlos Martin, Universidad de Zaragoza, Zaragoza, Spain; Dr Sheldon Morris, FDA/CBER, Bethesda, MD, USA; Dr Volker Öppling, Paul Ehrlich Institut, Langen, Germany; Dr Micha Roumiantzeff, Lyon, France; Dr. Masaaki Seki, Japan BCG Laboratory, Tokyo, Japan; Dr Keigo Shibayama, National Institute of Infectious Diseases, Tokyo, Japan; Dr James Southern, Advisor to Medicines Control Council in South Africa, Capetown, South Africa; Mr Apichai Supasansatorn, Ministry of Public Health, Nonthaburi, Thailand; Dr Yolanda Lopez Vidal, Universidad Nacional Autonoma de Mexico, Copilco-Universidad, Mexico City, Mexico; Dr Sri Wahyuningsih, National Agency of Drug and Food Control (NADFC), Jakarta Pusat, Indonesia; Dr Kenneth Barry Walker, NIBSC, Potters Bar, UK; Dr Saburo Yamamoto, Japan BCG Laboratory, Tokyo, Japan; Dr Zihou Yan, Chengdu Institute of Biological Products, Chengdu, P. R. China; Dr Lei Zhang, Chengdu Institute of Biological Products, Chengdu, P. R. China; Mrs AiHua Zhao, National Institute for the Control of Pharmaceutial & Biological Products, Beijing, P.R. China; Dr Hye-Na Kang and Dr Ivana Knezevic, QSS/IVB/FCH, WHO, Geneva, Switzerland.

Since then, several draft recommendations were prepared by Dr Mei Mei Ho, NIBSC, Potters Bar, UK with support from the drafting group, Dr Mike Corbel, Milton Keynes, UK; Dr Roland Dobbelaer, Lokeren, Belgium; Dr James Southern, Capetown, South Africa; Dr Kenneth Barry Walker, NIBSC, Potters Bar, UK; Dr Hye-Na Kang, QSS/IVB/FCH, WHO, Geneva, Switzerland.

Following the meeting of the drafting group in March 2011, Potters Bar, UK, draft recommendations were updated taking into account the comments received from:

Dr Murielle Andre, Agence Française de Sécurité Sanitaire des Produits de Santé, Lyon, France; Dr Miliana Chouchkova, National Center of Infectious and Parasitic Diseases, Sofia, Bulgaria; Dr Hazel Dockrell, London School of Hygiene and Tropical Medicine, London, UK; Dr Sunil Gairola, Serum Institute of India, Hadapsar, India; Dr Peter Hubrechts, Statens Serum Institut, Copenhagen, Denmark; Jeewon Jung, Korea Food and Drug Administration, Chungchengbuk-do, Republic of Korea; Dr Micheline Lagranderie, Institut Pasteur, Paris, France; Professor Diana Levi, Tarassevich State Research Institute for Standardization and Control of Medical Biological Preparations, Moscow, Russian Federation; Dr Volker Öppling, Paul Ehrlich Institut, Langen, Germany; Gandjar Trisnasari, Bio Farma, Bandung, Indonesia; Dr Yolanda Lopez Vidal, Universidad Nacional Autonoma de Mexico, Facultad de Medicina, Mexico City Mexico; Dr Veronique Vincent, Institut Pasteur, Paris, France; Dr Sri Wahyuningsih, National Agency of Drug and Food Control (NADFC), Jakarta Pusat, Indonesia; Dr Tang Ying, China National Biotec Group, Beijing, China; Mrs AiHua Zhao, National Institute for the Control of Pharmaceutial & Biological Products, Beijing, P.R. China.

The draft recommendations were posted on the WHO biologicals web site for public consultation from 1 to 23 June 2011.

The WHO/BS/2011.xxxx document was prepared by Dr Mei Mei Ho, NIBSC, Potters Bar, UK; Dr Hye-Na Kang, QSS/IVB/FWC, WHO, Geneva, Switzerland; Dr James Southern, Capetown, South Africa; Dr Kenneth Barry Walker, NIBSC, Potters Bar, UK, taking into account comments received from the following reviewers:

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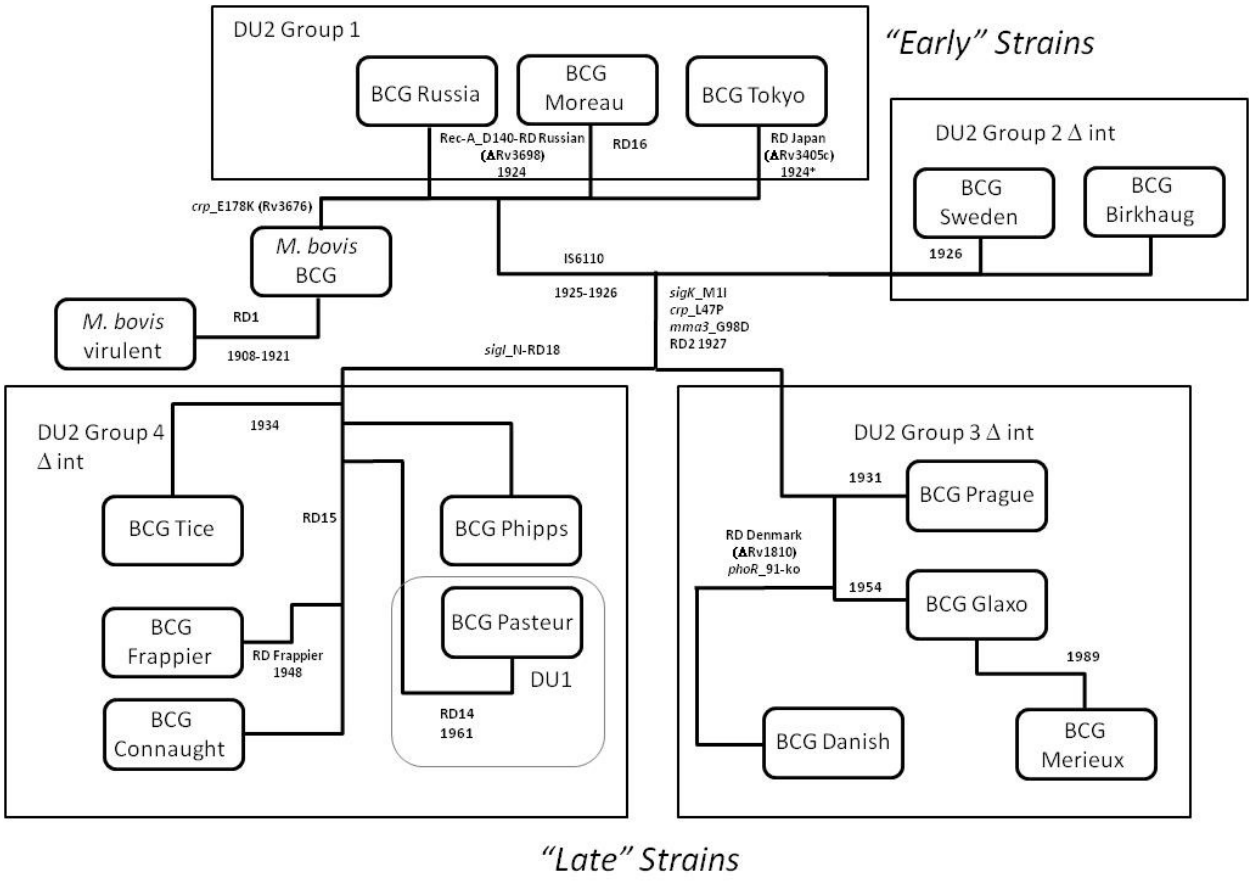
Appendix 1

History and genealogy of BCG sub-strains



Note: This diagram only provides information on a historical overview of the use of different sub-strains derived from BCG vaccine strain. It does not indicate any WHO "qualification" or "approval" of the strains or vaccines in the context of this document.

*Yamamoto S, Yamamoto T. Historical review of BCG vaccine in Japan. *Japanese Journal of Infectious Disease*, 2007, 60:331-336.

Appendix 2

Model summary protocol for manufacturing and control of BCG vaccine

The following protocol is intended for guidance, and indicates the information that should be provided as a minimum by the manufacturer to the NRA.
Information and tests may be added or deleted as required by the NRA, if applicable.

It is thus possible that a protocol for a specific product may differ in detail from the model provided. The essential point is that all relevant details demonstrating compliance with the license and with the relevant WHO recommendations of a particular product should be given in the protocol submitted.

The section concerning the final product must be accompanied by a sample of the label and a copy of the leaflet that accompanies the vaccine container. If the protocol is being submitted in support of a request to permit importation, it must also be accompanied by a lot release certificate from the NRA or national control laboratory of the country in which the vaccine was produced stating that the product meets national requirements as well as Part A recommendations of this document published by WHO.

Summary information on the finished product (final lot)

International name	_____
Trade name	_____
Product licence (marketing authorization) number	_____
Country	_____
Name and address of manufacturer	_____
Site of manufacture of final lot	_____
Name and address of licence holder if different	_____
BCG sub-strain	_____
Authority that approved BCG sub-strain	_____
Date approved	_____
Final bulk number	_____
Volume of final bulk	_____
Final product	
Type of vaccine	**Intradermal/ Percutaneous/ Other**
Final lot number	_____
Type of container	_____
Number of doses per container:	_____
Number of filled containers in this final lot	_____

Date of manufacture of final lot _____

Date on which last determination of bacterial count was started or date of start of period of validity _____

Shelf-life approved (months) _____

Expiry date _____

Diluent _____

Storage conditions _____

Volume of single human dose _____

Volume of vaccine per container _____

Number of doses per container _____

Summary of the composition (Include a summary of the qualitative and quantitative composition of the vaccine per human dose _____

Release date _____

Production information

A genealogy of the lot numbers of all vaccine components used in the formulation of the final product will be informative.
The following sections are intended for the reporting of the results of the tests performed during the production of the vaccine, so that the complete document will provide evidence of consistency of production; thus if any test has to be repeated, this must be indicated. Any abnormal results should be recorded on a separate sheet.

Control of source materials (A.3)

The information requested below is to be presented on each submission. Full details on master and working seed-lots upon first submission only and whenever a change has been introduced.

Master seed lot

Origin of seed lot _____

Master seed lot number. _____

Name and address of manufacturer _____

Passage level _____

Date of preparation of seed lot _____

Date of receipt of seed lot (if applicable) _____

Date of reconstitution of seed lot ampoule _____

Date approved by the National Regulatory Authority: _____

Working seed lot

Working seed lot number. _____

Name and address of manufacturer _____

Passage level _____

Date reconstitution of seed lot ampoule _____

Date approved by the National Regulatory Authority _____

Tests on working seed lot production (A.3.2)

Identity test (A.3.2.1)

Method used _____

Date test start _____

Date test complete _____

Results _____

Test for bacterial and fungal contamination (A.3.2.2)

Method used _____

Number of containers tested _____

Volume of inoculum per container _____

Volume of medium per container _____

Observation period (specification) _____

Incubation	Media used	Inoculum	Date test start	Date test complete	Results
20–25 °C	_____	_____	_____	_____	_____
30–36 °C	_____	_____	_____	_____	_____
Negative control	_____	_____	_____	_____	_____

Test for absence of virulent mycobacteria (A.3.2.3)

Method used _____

No. of human dose injected per guinea-pig _____

Inoculation route _____

No. of guinea-pigs given injection _____

Weight range of guinea-pigs _____

Observation period (specification) _____

Date test start _____

Data test complete _____

Health of animals during test _____

Weight gains (losses) _____

Result _____

Test for excessive dermal reactivity (A.3.2.4)

	vaccine	**reference vaccine**
Method used	_____	_____

Dilutions injected	_____	_____
Inoculation route	_____	_____
No. of guinea-pigs given injection	_____	_____
Observation period (specification)	_____	_____
Date test start	_____	_____
Data test complete	_____	_____
Mean diameter of lesions (for each dilution)	_____	_____
Result	_____	_____

Production of culture medium (A.3.3)

Any components of animal origin _____

Certificate for BSE/TSE-free _____

Control of vaccine production (A.4)

Control of single harvests (A.4.1)

Derived from master seed lot number. _____

Working seed lot number _____

Passage level from master seed _____

Culture medium

Number and volume of containers inoculated

Date of inoculation _____

Temperature of incubation _____

Date of harvest _____

Results of visual inspection _____

Control of final bulk (A.4.2)

Tests for bacterial and fungal contamination (A.4.2.2)

Method used _____

Number of containers tested _____

Volume of inoculum per container _____

Volume of medium per container _____

Observation period (specification) _____

Incubation	Media used	Inoculum	Date test start	Date test complete	Result
20°–25°C	_____	_____	_____	_____	_____
30°–36°C	_____	_____	_____	_____	_____
Negative control	_____	_____	_____	_____	_____

Test for absence of virulent mycobacteria (A.4.2.3) (if test not performed on final lot)

Method used _____

No. of human dose injected per guinea-pig _____

Inoculation route _____

No. of guinea-pigs given injection _____

Weight range of guinea-pigs _____

Observation period (specification) _____

Date test start _____

Data test complete _____

Health of animals during test _____

Weight gains (losses) _____

Result _____

Test for bacterial concentration (A.4.2.4)

Method used _____

Date test start _____

Data test complete _____

Specification _____

Result _____

Test for number of culturable particles (A.4.2.5)

Method used _____

Date test start _____

Data test complete _____

Specification _____

Result _____

Information of working reference preparation _____

Substances added (A.4.2.6)

Any components of animal origin _____

Certificate for BSE/TSE-free _____

Filling and containers (A.5)

Lot number _____

Date of filling _____

Volume of final bulk filled _____

Filling volume per container _____

Number of containers filled (gross) _____

Date of freeze-drying _____

Number of containers rejected during inspection _____

Number of containers sampled _____

Total number of containers (net) _____

Maximum period of storage approved _____

Storage temperature and period _____

Control tests on final lot (A6)

Inspection of final containers (A.6.1)

Appearance _____

Date of test _____

Specification _____

Result _____

Recommended reconstitution fluid _____

Volume of reconstitution fluid per final container _____

Identity test (A.6.2)

Method used _____

Date test start _____

Date test complete _____

Specification _____

Result _____

Tests for bacterial and fungal contamination (A.6.3)

Method used _____

Number of containers tested _____

Volume of inoculum per container _____

Volume of medium per container _____

Observation period (specification) _____

Specification _____

Incubation	Media used	Inoculum	Date test start	Date test complete	Result
20°–25°C	_____	_____	_____	_____	_____
30°–36°C	_____	_____	_____	_____	_____
Negative control	_____	_____	_____	_____	_____

Safety tests (A.6.4)

Test for absence of virulent mycobacteria (A.6.4.1) (if test not performed on final bulk)

Method used _____

No. of human dose injected per guinea-pig _____

Inoculation route _____

No. of guinea-pigs given injection _____

Weight range of guinea-pigs _____

Observation period (specification) _____

Date test start _____

Data test complete _____

Health of animals during test _____

Weight gains (losses) _____

Specification _____

Result _____

Test for excessive dermal reactivity (A.6.4.2) if applicable

	vaccine	**reference vaccine**
Method used	_____	_____
Dilutions injected	_____	_____
Inoculation route	_____	_____
No. of guinea-pigs given injection	_____	_____
Observation period (specification)	_____	_____
Date test start	_____	_____
Data test complete	_____	_____
Mean diameter of lesions (for each dilution)	_____	_____
Specification	_____	_____
Result	_____	_____

Test for bacterial concentration (A.6.5)

Method used _____

Date test start _____

Data test complete _____

Specification _____

Result _____

Test for residual moisture (A.6.6)

Method _____

Date _____

Specification _____

Result _____

Tests for viability (A.6.7)

Test for number of culturable particles (A.6.7.1)

Method used _____

Medium _____

Date test start	_____	
Data test complete	_____	
	Before lyophilization	**After lyophilization**
No. of containers tested	_____	_____
Mean count of culturable particles per mL	_____	_____
Mean survival rate (%)	_____	_____
Specification	_____	
Result	_____	
Information of working reference preparation	_____	

Rapid test for viability (A.6.7.2) if applicable

Method	_____
Mean survival rate (%)	_____
Date	_____
Specification	_____
Result	_____

Thermal stability test (A.6.8)

Method used	_____	
Date test start	_____	
Data test complete	_____	
	Unheated containers	**Heated containers**
No. of containers tested	_____	_____
Culturable particles in each container per mL	_____	_____
Mean survival rate (%)	_____	_____
Specification	_____	
Result	_____	
Information of working reference preparation	_____	

Submission addressed to national regulatory authority

Name of responsible person (typed) _____

Certification by the person from the control laboratory of the manufacturing company taking over responsibility for the production and control of the vaccine:

I certify that lot no. _____ of BCG vaccine, whose number appears on the label of the final container, meets all national requirements and/or satisfies Part A of the Recommendations for Biological Substances No. *3* (Recommendations for BCG vaccine, revised 2011)

Signature: _____

Name (typed): _____

Date: ____

Appendix 3
Model certificate for the release of BCG vaccine by national regulatory authorities

LOT RELEASE CERTIFICATE

The following lot(s) of BCG vaccine produced by _____[(1)] in _____[(2)], whose numbers appear on the labels of the final containers, meet all national requirements[(3)] and Part A[(4)] of the WHO recommendations to assure the quality, safety and efficacy of freeze-dried BCG vaccines (_____)[(5)], and comply with Good Manufacturing Practices for Pharmaceutical Products: Main Principles[(6)] and Good Manufacturing Practices for Biological Products[(7).]
As a minimum, this certificate is based on examination of the summary protocol of manufacturing and control.

The certificate may include the following information:

- Name and address of manufacturer;
- Site(s) of manufacturing;
- Trade name and/common name of product;
- Marketing authorization number;
- Lot number(s) (including sub-lot numbers, packaging lot numbers if necessary);
- Type of container;
- Number of doses per container;
- Number of containers/lot size;
- Date of start of period of validity (e.g. manufacturing date) and/or expiry date;
- Storage condition;
- Signature and function of the authorized person and authorized agent to issue the certificate;
- Date of issue of certificate; and
- Certificate number.

The Director of the National Regulatory Authority (or Authority as appropriate):

Name (Typed)
Signature
Date

[1]Name of manufacturer
[2]Country of origin
[3]If any national requirements are not met, specify which one(s) and indicate why release of the lot(s) has nevertheless been authorized by the NRA.

[4]With the exception of provisions on distribution and shipping, which the NRA may not be in a position to assess.

[5]WHO Technical Report Series, No. ___, YYYY, Annex __.

[6]WHO Technical Report Series, No. 908, 2003, Annex 4.

[7]WHO Technical Report Series, No. 822, 1992, Annex 1.

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